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Review Article

An array of various microbiological diagnostic modalities for pulmonary tuberculosis: A review

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ABSTRACT

Pulmonary tuberculosis (TB) is a public health concern in India and rapid diagnostic and cutting-edge methods are needed to identify its drug-resistant forms. The National Strategic Plan 2017-2025 aims for a "TB-Free India" by 2025, based on the 'Detect, Treat, Prevent, and Build' pillars. The aim of this review was to present an updated overview of current initiatives to improve traditional diagnostic techniques as well as novel molecular methods for diagnosis of pulmonary tuberculosis patients.

The studies were scanned for published publications on tuberculosis, Mycobacterium, TB culture, and other methodologies using a range of scientific sources, including PubMed and Google Scholar. Although a plethora of diagnostic tests are available for pulmonary tuberculosis, each has limitations and advantages. Nonetheless, a combination of tests allows us to obtain an accurate diagnosis more quickly, which helps us to treat these individuals effectively.

Rapid reporting and treatment decisions are necessary for tuberculosis diagnosis; however, there are issues with the current approaches- Certain individuals may receive false positive findings, including those who have received prior BCG vaccinations, young people infected with Mycobacterium tuberculosis (MTB), and people coinfected with HIV. The World Health Organization (WHO) recommends next-generation Nucleic Acid Amplification Tests (NAATs) for point-of-care diagnostic healthcare settings, such as GeneXpert MTB/RIF (GX), and proposes POC-NAATs (2nd generation) for TB detection in the future.

1. Introduction

In 2003, the global burden of tuberculosis (TB) exceeded 1.7 billion; the prevalence of TB was high globally and appeared to be steadily decreasing. According to estimates from the WHO 2021, 10.6 million people were diagnosed with tuberculosis, and 1.6 million of those cases resulted in death.[1]

In India, the incidence of Mycobacterium tuberculosis is a common and serious health issue. The National Strategic Plan 2017-2025 was built on the four pillars of 'Detect-Treat-Prevent-Build' for universal coverage and social protection, which was launched in 1997 and renamed the RNTCP (Revised National TB Control Program) to the NTEP (National Tuberculosis Elimination Program), aiming to achieve a "TB Free India" by the year 2025. This program is designed to reduce the incidence of and mortality from tuberculosis (TB) and is five years ahead of the Sustainable Development Goals Target. The quick, automated, cartridge-based nucleic acid amplification test (NAAT) is used to determine whether a disease is present.[2–5]

The WHO recommends using tuberculosis screening tests to identify patients who may be at risk and to quickly diagnose and start treating pulmonary tuberculosis. Systematic TB diagnostic methods, such as radiological

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diagnosis, microscopy, BACTEC Mycobacterium Growth Indicator Tube (MGIT) 960 system, line probe assay (LPA), lateral flow urine lipoarabinomannan assay (LF-LAM) and molecular testing, include Xpert MTB/RIF and loop-mediated isothermal amplification (LAMP).[6–8] Accurately and quickly diagnosing tuberculosis (TB) can still be difficult, even with the availability of multiple sophisticated diagnostic tests, particularly in highly endemic patients.[9–14] This review provides an outline on the diagnostic tools for Mtb including identification and detection of mycobacteria using conventional methods as well as new strategies based on host response to Mtb, which have been tested for TB diagnosing.

2. Search Strategy

Scientific databases, including PubMed and Google Scholar, were searched to find published studies. A literature search was performed by using terms such as LAMP, Tuberculosis, Mycobacterium, and Mycobacteria in a variety of combinations without any restrictions: Mycobacterium" or "LAMP, 'TB Culture', 'Xpert MTB/RIF', 'Line Probe Assay (LPA', 'Micro Real-Time PCR', 'Antigen Detection (MPT 64)', 'Tuberculin Skin Testing (TST)', 'Interferon-Gamma (IFN-γ) Release Assays (IGRAs)', 'Next-Generation Sequencing (NGS)', and 'Raman Spectrometry'. To identify relevant papers, we reviewed the references in the original research that were included and had already been published.

3. Analysis of the Literature

3.1. Mycobacterium tuberculosis diagnosis

A combination of tests enables us to reduce the turnaround time and achieve optimal diagnosis, leading to proper management of these patients (Table 1).

3.2. Microscopy

Acid-fast bacilli (AFB) can be easily and inexpensively identified by direct microscopy. Sputum and gastric aspirate smear microscopy were used for conventional procedures in developing countries for diagnosing pulmonary tuberculosis. Ziehl-Neelsen (Z-N) staining methods frequently use a carbol fuschin solution.[17]

Mycobacterium tuberculosis resists decolonization with acid-containing reagents. Due to the lipid-rich cell wall, microscopic examination of acid-fast organisms in smears from sputum and alveolar lavage fluid is possible. The effectiveness of microscopy has been validated by international studies, yielding sensitivities and specificities ranging from 25.3–81.6% and 83.4–99%, respectively.

The lack of sensitivity is the main limitation of smear microscopy; it varies significantly (20 to 80%) across studies and is unsatisfactory in pediatric TB patients.[18] Light-emitting diode (LED) microscopy and mercury vapor fluorescence are two techniques that have largely replaced conventional ZN staining as methods to increase efficacy.[19] Considering that light-emitting diodes (LEDs) are sustainable and have a lifespan that is greater than that of powerful light sources, LED microscopy can be used in environments with limited resources. Therefore, the WHO recommends that LED microscopy take over the role of conventional fluorescence microscopy.[1,20]

In addition to increasing efficiency, cutting expenses and labor, fluorescence microscopy can increase the sensitivity of smear microscopy. Due to the nonspecific nature of fluorochrome dye incorporation, fluorescence microscopy may yield false-positive results.[21] There have been instances of instability in fluorescence staining.[22] In regions with a high TB burden, sputum smear microscopy is a reasonably quick, affordable, and specific method for diagnosing MTB. Consequently, it is still a valuable technique for diagnosing MTB, particularly in nations with limited resources. The primary constraint of microscopy pertains to its inadequate sensitivity in diagnosing tuberculosis, particularly in cases involving paucibacillary specimens.

3.2.1. ReaSLR method

The ReaSLR technique is an affordable and simple method for processing sputum samples for tuberculosis diagnosis. It involves fast liquefaction with ReaSLR reagent, filtration, centrifugation, and sediment use for smear microscopy. A study at the Sanjay Gandhi Institute of Medical Sciences in Lucknow, India, evaluated 150 sputum samples from patients with pulmonary TB. The ReaSLR method achieved a higher percentage of positive results (31.33% versus 12%) and a higher sensitivity (90.47% vs. 91.6%).[23,24]

3.3. Culture

3.3.1. Solid and liquid culture

The TB culture method is more sensitive than smear microscopy, which requires more than 5000 AFB/ml to yield a positive finding. However, all AFBs definitively confirmed the infection. The MTB culture method was used for confirmation of TB infection.[25] Solid culture is a less susceptible and cost-effective method, while liquid culture is more convenient, has high sensitivity, and is faster due to automatic growth detection.

Tubercle bacilli can be grown using Ogawa's media, egg-based media, or Lowenstein-(L-J) Jensen's media.[26] Glycerol, which is present in L-J media, promotes the development of Mtb but not Mycobacterium bovis, and sodium pyruvate increases the growth of M. bovis. The sensitivity of the Middlebrook 7H9 culture test was 76% (95% CI, 0.69–0.83). In comparison, the Ogawa test had the lowest sensitivity at 63% (95% CI 0.55-0.71).[11,27]

The WHO advises employing both systems when it is practical. Liquid-based systems have the main benefit of

Table 1: The efficacy of various tests for pulmonary tuberculosis has been reported by different researchers.

Test	Principle/ Technology	Sensitivity (%)	Specificity (%)	Target setting	Comments
Xpert MTB/RIF	NAAT qPCR	88	98	District or sub districtlabs	Limited utility in resource-limited settings [6]
Sputum smear microscopy	Ziehl-Neelsen staining and microscopic detection of bacilli	25-82	83-99	Peripheral and reference labs	Cannot differentiate Mtb and other AFB [9]
LED- fluorescence method	Auramine staining and detection by fluorescence microscope	52-97	94-100	Peripheral and reference labs	[10]
Lowenstein Jensen (LJ)	identification by colony morphology & biochemical tests	63	>99	Secondary & tertiary centers	Longer turnaround time [11]
BACTEC Mycobacterium Growth Indicator Tube (MGIT) 960 system	Liquid culture method with drug susceptibility testing	76	>99	reference labs	Mtb identification requires additional laboratory tests [11]
Tuberculin skin test (TST)	Host immune reaction to Mtb in the body	87–98	74–96	Secondary & tertiary centers	False +ve in BCG vaccinated, NTM infected & high endemic regionsLow sensitivity in immune-compromised individuals [10, 12]
Loop-mediated isothermal amplification (LAMP)	NAAT	93	94	Reference labs	Simple method to use in resource-limited settings & high endemic regions [14]
Serological tests (Antigen)	Detection of Mtb mycolic acid components & inflammatory biomarkers	76	100	Peripheral & reference labs	Results may vary depending on host metabolic and disease states [15]
Interferon-gamma release assay (IGRA)	Immune response against Mtb antigen	(T Spot) 90 (QFT) 94	(T Spot) 91 (QFT) 93	Secondary & tertiary centers	Not recommended to predict active TB and treatment decision [13 16]

having a short detection time; the mean time to detection is 12.8 days, while for solid-based media, it varies from 25.1 to 25.5 days. [28] However, their practical utility can be restricted by insufficient laboratory facilities in locations with limited resources.

3.3.2. Culture-based drug sensitivity test (DST)

The primary benefit of liquid culture is that it grows rapidly, which has increased the use of methods based on liquid broth, such as the MGIT. The fully automated BACTEC MGIT 960 system produces results in 2 weeks. [29] Currently, determining drug resistance persists, as "culture-based DST remains the gold standard". [30] The two techniques that are currently in use are critical concentration and minimum inhibitory concentration (MIC) methods. The critical concentrations are often updated, allowing increased correlations between phenotypic and genotypic sensitivity results. A recent decrease in the threshold concentration is necessary to assess RIF resistance. [31,32] The MIC was the lowest, resulting in no visible bacterial growth. [33]

Ongoing research and improvements in diagnostic methods, such as the development of novel culture methods and the integration of automated systems, are crucial for addressing these challenges. Additionally, exploring the potential of alternative diagnostic techniques, such as transcriptomics, can further enhance the accuracy and efficiency of Mtb diagnosis. [34] It is evident that a multifaceted approach is necessary to effectively diagnose and manage tuberculosis, ensuring timely treatment and prevention of further transmission.

3.3.3. Rapid identification from positive cultures

Considering the short turnaround time of usual biochemical testing (2 to 3 weeks), rapid identification assays identify Nontuberculous mycobacterial pulmonary disease and *Mycobacterium tuberculosis* complex after positive

cultures, enabling early anti-TB medication.[35] The Mtb protein 64 (MPT-64) is secreted during bacterial growth and is unique to Mtb. Enzyme-linked immunosorbent assays (ELISAs), a crucial part of immunochromatographic (ICT) tests, detect the MPT-64 antigen through double sandwiches.[36] For rapid identification of Mtb complexes, a study revealed that ICT tests have high sensitivity, ranging from- 98.1 to 98.6%, and high specificity, ranging from 99.2 to 100%.[37]

3.4. Approaches based on molecular biology

3.4.1. Xpert MTB/RIF

Many studies conducted in resource-limited settings have focused on the diagnostic accuracy of the Xpert MTB/RIF assay.[38,39] The "Foundation for Innovative and New Diagnostics" report was published in 2010. At four research sites in India, Peru, South Africa and Azerbaijan, information regarding "1730 individuals who were suspected of having drug-resistant and drug-sensitive tuberculosis" was available.

Sputum samples from 561 (98.2%) smear-positive patients and 124 (72·5%) smear-negative patients were tested directly, yielding results for 551 (98·2%) of the total tuberculosis patients. Of the 609 patients who did not have tuberculosis, 604 (99.2%) had a positive test result. The use of morning samples, spot samples, and morning + spot samples was associated with sensitivities of 72·5%, 85·1%, and 90·2%, respectively, for smear-negative patients.[39] The turnaround time of the diagnosis procedure was 1 day for microscopy, 6–16 days for liquid culture (Middle Brook), and 8 weeks for solid culture (L–J medium).[40,41] Xpert Ultra, the total sensitivity increased from 83% to 88% as a result of these changes, although its specificity slightly decreased from 98% to 96%.[6,42]

3.4.2. Loop mediated isothermal amplification (LAMP)

LAMP was first described by Notomi (2015), who employed DNA polymerase and unique primers that can recognize various target DNA sequences. The final product of the reaction involves multiple repetitions and inversions of the target DNA copies, resulting in 10^9 copies within an hour.[43] LAMP offers numerous advantages over PCR, including visual identification through gel electrophoresis and real-time PCR, making it a more efficient method for detecting diseases.

This isothermal reaction, which has a high detection limit and specificity, can amplify DNA from a crude sample and can be visualized using the naked eye, gel electrophoresis, or turbidometry. The procedures he mentioned are simple and highly specific.[14] The sensitivity and specificity of the LAMP assay were 93% and 94%, respectively. Thus, in systems with limited resources, since the TB-LAMP assay performs better in terms of diagnosis than smear microscopy, the World Health Organization currently recommends using it instead.[1]

3.4.3. Line probe assay (LPA)

LPA is a polymerase chain reaction (PCR) technique that is used to- detect genetic mutations and MTB complexes associated with drug resistance.[44] The basic procedure for LPA is to allow the DNA probe to solidify on the membrane strip, collect the previously labeled amplification product, and use colorimetry to assess the result. The basic procedure for LPA is to allow the DNA probe to solidify on the membrane strip, collect the previously labeled amplification product, and use colorimetry to assess the result. The output of the LPA is seen as a linear band.[45] Other commercial tests capable of detecting resistance to INH and RIF for first-line treatment, namely, the INNO-LiPA Rif TB Kit and GenoType MTBDRplus 1.0 by Inno genetics and Hain Life science, respectively, have been developed.[46]

The more recent version of LPA, known as GenoType MTBDRplus 2.0, has increased sensitivity in identifying Mtb strains in both smear-positive and smear-negative specimens. Genotype MTBDRsl from Hain Lifesciences can be employed to identify mutations associated with second-line drugs, namely, capreomycin, kanamycin, and amikacin, as well as fluoroquinolones.[47]

3.4.4. Micro real-time PCR

The Truelab portable platform reports outcomes in less than one hour using micro real-time PCR-based chip-based assays for tuberculosis detection and Truenat MTB, Truenat MTB Plus, and Truenat MTB-Rif Dx tests.The reference used went to the extent of inviting the WHO to convene in December 2019 to propose standardized use cases for Truenat assays and other rapid molecular tests. The correspondence suggests that, in the identification of TB and RIF treatment, the Truenat MTB, MTB Plus, and MTB-Rif Dx assays display similar sensitivities and specificities as Xpert MTB/RIF; as a result, clinicians' behavior would be the same regardless of which test was selected for infection post testing.

The typical sensitivities of Truenat MTB and Truenat MTB Plus were 73% and 80%, respectively. The sensitivity of the Truenat MTB-RIF test was 84%. Even though the Truenat test seemed to have high specificity; according to head-to-head evaluations performed by central reference laboratories, the performance of the Truenat test seemed to be similar to that of the Xpert MTB/RIF.Compared with microbiological reference standards, Truenat MTB-Rif Dx had a sensitivity and specificity of 57.1% and 91.8%, respectively. Truenat MTB-RIF Dx had a sensitivity and specificity of 58.7% and 87.5%, respectively, while GeneXpert MTB/RIF had a sensitivity and specificity of 56% and 91.4%, respectively.[3–5,48]

3.5. Immunodiagnostic approaches

3.5.1. Antibody detection

Serological tests based on the humoral immune response to antibodies against the antigens of *Mycobacterium tuberculosis* identify the disease.Nevertheless, the criteria are characterized by poor performance, including low sensitivity and specificity for the diagnosis of pulmonary TB. At the same time, the World Health Organization indicates that no commercial serologic assays are recommended for the diagnosis of tuberculosis.[49]

3.5.2. Antigen detection

Rapid immune-chromatography for detecting tuberculosis antigens is one of the latest technologies. This test was used to detect the antigens secreted by *Mycobacterium tuberculosis*, which are the culture filtrate protein, *Mycobacterium tuberculosis* protein, and proteins encoded by gene regions with differences in RDI, RD2, RD3 and the secreted antigenic target 6 kDa protein. The sensitivity and specificity of the test for detecting the fast antigen TB MPT64 were 75.61% and 100%, respectively. The TB MPT64 antigen had a 100% positive predictive value and 78.72% negative predictive value when used to calculate the negative predictive value (NPN) of the TB MPT64 test.[7,50]

3.5.3. Tuberculin skin testing (TST)

The pure protein derivative (PPD) of tuberculin is used in the TST, a conventional method, for identifying type IV hypersensitivity. Activated lymphocytes that can recognize MTB antigens can be found in patients who have MTB infection.[51] When Mtb antigens stimulate sensitized T cells, soluble lymphokines are released to increase induration, edema, localized redness, and vascular permeability. Tuberculin, a pure protein derivative (PPD), is injected intradermally. Forty-eight to 72 hours later, induration at the injection site (≥5 mm is considered positive) is assessed to determine an individual's immunological response. The precision of the TST outcome is contingent upon proficient individuals administering PPD intradermally.[15]

The test is suitable for detecting MTB exposure in geographic locations with rare TB patients. People with nontuberculous mycobacterial infections, those from highly endemic locations, and those who have received Bacillus Calmette Guerin (BCG) immunization may experience false-positive test results. TST sensitivity is reduced in people with impaired immune systems.[12,52]

3.5.4. Interferon-gamma (IFN-γ) release assays (IGRAs)

IGRAs are based on the release of IFN-γ from lymphocytes exposed to Mtb-specific antigens (TBAg), including early-secreted antigenic target 6, culture filtrate protein 10, and CFP-10, in the intraepithelial mucosa TB ESAT-6. BCG vaccination does not affect GGR results. The reason for the positive detection of MTB infection was NTM, which is more specifically TST. The two commercial type T-spot and Quanti FERON-TB test kits are currently available: Qiagen; T-spot, Oxford Immuno-tech TBC.[16]

3.5.5. Spot

The results showed that the T-SPOT could also be used for prospective screening of immigrant groups, medical personnel, and university students. Upon activation with an Mtb-specific antigen, the T-SPOT assay revealed several IFN-γ-secreting cells. The enzyme-linked immunospot method serves as its foundation. In addition, MTB infection was identified in pediatric and HIV patients.[53]

The ratio of TB-specific antigen: phytohemagglutinin: TBAg/PHA ratio is mandated, likely given the ESAT-6/PHA and C antigens/PHA of the T-SPOT assay. Our biological data illustrate several alternatives to utilizing the T-SPOT assay to increase its precision in detecting active TB. Specifically, immunocompromised active TB patients have reduced TBAg.

This approach was used to distinguish active TB from LTB with weak immunity connected with LTBI due to the current low TBAg.[54] As a measure of the host's immunological health, the use of PHA, the positive control for the "T-SPOT", is limited. Mean spot sizes (MSS): In the "T-SPOT assay", the MSS of ESAT-6" spot-forming cells was calculated by an automated ELISPOT reader. The specificity and sensitivity were 91.02% and 90.12%, respectively.[13]

3.5.6. QFT

An ELISA was used in the QFT experiment to identify IFN-γ generated in the culture medium supernatant following Mtb-specific antigen stimulation.[55] A fourth-generation QuantiFERON-TB Plus assay (QFT-Plus), a TB antigen tube that contains more truncated peptides from ESAT-6 and CFP-10, was used to discover the CD4+ and CD8+ responses of T cells and was generated with the hope of enhancing the identification of these traits in immunosuppressed facilities. Moreover, interferon-γ-producing CD8+ T- cells specialized for M. tuberculosis have been detected in energetic instances of tuberculosis individuals who are coinfected with HIV and in young people with tuberculosis. Previous studies have reported that the sensitivity and specificity of the QFT-PLUS were 94% (95% CI 92-96%; I2 = 86.7%) and 93% (95% CI 91-95%; I2 = 78.6%), respectively.[55]

3.6. New techniques

3.6.1. Next-generation sequencing (NGS)

As it generates data much faster than traditional phenotypic culture or culture-based testing, NGS, or next-generation sequencing, is considered a viable alternative for comprehensive DSTs for pulmonary tuberculosis.Although state-of-the-art molecular tests rely on thousands of

probes, they are restricted to probe-specific targets. Through multigene region sequencing and whole-genome sequencing, NGS can provide accurate labeled sequence information for complete genomes. The WHO has published guidelines on the utilization of NGS technology for the interpretation and identification of mutations associated with phenotypic drug resistance in the Mtb complex compiles.[56]

ReSeqTB was an initiative established by the WHO in 2019 that was intended to serve as a TB sequencing database, sorting and standardizing DR-TB pertaining to information with genotypic and phenotypic DSTs.[8] Numerous stakeholders are trying to prove that targeted sequencing may be a full end-to-end DR-TB diagnosis solution.

The methods used for targeted library preparation and sequencing, as well as results reported (direct DNA extraction from respiratory samples without culturing), are all included in this study.(Diagram 1) At least seven countries have already effectively used sequencing for DR-TB surveillance: the Philippines, South Africa, Azerbaijan, Bangladesh, Belarus, Pakistan, and Ukraine. In addition, India has recently voiced interest in potentially adopting a sequencing-based approach for drug susceptibility surveillance.[57,58] Gaps and potentially future initiatives for genotypic drug susceptibility testing and TB diagnosis (Diagram 2).

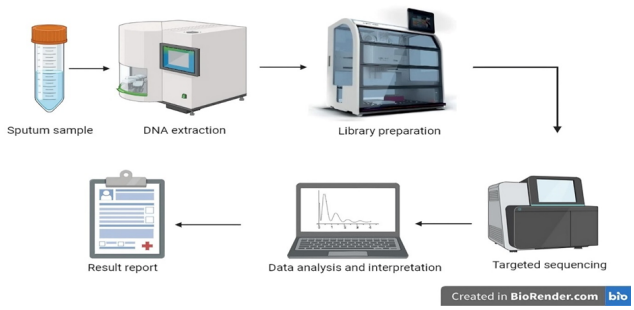

Diagram 1: Schematic workflow representation of targeted sequencing

The WHO's updated module-3 on tuberculosis in 2024 revealed that three products met the inclusion criteria for drug resistance detection to at least one of the anti-TB drugs under evaluation.[59]

The *Deeplex® Myc-TB* test is a NGS-based kit for identifying mycobacterial species, genotyping, and predicting drug resistance in *mycobacterium tuberculosis* (MTBC) strains. It uses deep sequencing to target 18 MTBC gene regions associated with anti-TB drug resistance. The test uses Nextera XT and DNA Flex library preparation kits and an automated analysis pipeline.[59]

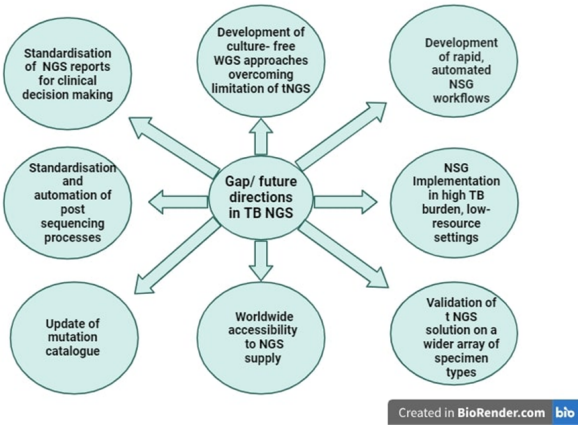

Diagram 2: Gaps and future directions in NGS for tuberculosis diagnosis

The *AmPORE-TB* test is a targeted NGS-based kit used to identify mycobacterial species and detect MTBC genetic variants associated with antimicrobial resistance in DNA extracted from sputum samples. It uses a 27-plex amplicon mix, including drug-resistant targets, genotyping targets, non-tuberculous mycobacteria (NTM) identification targets, and an internal control. The test is performed using the OND *AmPORE-TB* kit and Flow Cells on the GridION Diagnostic Sequencing System.[59]

The TBseq® test is a targeted NGS kit used for identifying mycobacterial species and predicting drug resistance in MTBC strains. It targets 21 genes associated with anti-TB drug resistance and is applicable to clinical specimens like sputum and bronchoalveolar lavage fluid. The assay includes automated analysis software and a secure online application.[59]

3.6.2. *Nanoparticle-based diagnostics*

Nano diagnostics uses nanotechnology for high-sensitivity, pre-infection detection of infections. Its nanoscale size and high surface-to-volume ratio make it indispensable in various fields. Robust, cost-effective, and reproducible platforms could be particularly useful for infectious disease diagnosis in developing countries.[60]

Gold nanoparticles (AuNPs) are ideal for clinical diagnosis, treatments, and multidisciplinary research due to their unique physiochemical and optical properties. They enhance antibody-antigen reactions, increasing test sensitivity and providing an easy, low-cost assay for multiple sample testing. AuNP probes offer a low-cost alternative for colorimetric detection of target genes from test DNA samples. AuNPs were first used in tuberculosis (TB) diagnosis, using DNA probes and AuNPs for colometric detection. The method is more accurate than InnoLiPA-Rif-TB, more sensitive than smear microscopy, and can be visualized for detection. It reduces contamination

chances and takes approximately 15 minutes per sample, making it a quick and efficient method.[61]

Colloidal AuNPs coated with M. tuberculosis antigen were used as a counter or detector reagent in an assay. The binding of serum samples or antibodies to the coated AuNPs was visually detected.

Mesoporous silica nanoparticles have been used in imaging, drug delivery, and biosensors. An indirect immunofluorescence microscopy method, SYBR Green I mediated assay, has been developed for the rapid detection of M. tuberculosis using bio-conjugated fluorescent silica nanoparticles.[62]

3.6.3. Raman spectroscopy

The diagnosis of pulmonary tuberculosis infection was made using Raman spectroscopy. A narrow-bandwidth laser beam stimulates the material in Raman spectroscopy. "Surface-enhanced Raman Spectroscopy (SERS) using a bead-beating module of a labon-chip (LOC) device" can be used to differentiate between nontuberculous mycobacteria and *Mycobacterium tuberculosis* complexes. The sample scatters light at different frequencies that are specifically associated with its molecular structure and attached functional groups, and these frequencies are known as Raman signature peaks.[63]

Blood and sputum are examples of bodily fluids that are known to produce specific biomarker molecules in a variety of illnesses. These biomarkers can be used to diagnose illnesses. One method is to look for variations in the Raman spectra that are brought on by the patient's fluids. Raman spectroscopy, which requires a short turnaround time and is inexpensive, has the ability to diagnose latent tuberculosis and active tuberculosis. The sensitivity and specificity of Raman spectroscopy for tuberculosis diagnosis are 91% and 94%, respectively.[64]

4. Conclusion

Early diagnosis and prompt initiation of effective anti-TB treatment remain the mainstays of successful implementation of NTEP by 2025 in India. A plethora of tests of varying sensitivity and specificity are available for diagnosis of tuberculosis, ranging from simple Z-N stain smear microscopy to highly complex next-generation sequencing, each having its advantages and limitations. The more important complex tests, such as the molecular biology and immunology approaches, gain more attention by researchers.

The present review gives a comprehensive description of various diagnostic modalities for tuberculosis. As long as the battle between Mycobacterium tuberculosis and human immune systems will be going on, the search for ever-more advanced and cost-effective, rapid point-of-care diagnostics will also ontinue which are appropriate to resource-constraint countries, including India.

5. Source of Funding

None

6. Conflicts of interest

None

7. Ethical Approval

Not Applicable

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